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ECONOMICAL AND RELIABLE ESTIMATES OF GENERAL COMBINING ABILITY FOR BLISTER RUST RESISTANCE OBTAINED WITH MIXED-POLLEN CROSSES[1]

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ABSTRACT

Two kinds of mixed-pollen crosses (using equal volumes of fresh pollen from either 4 or 10 trees) and a series of four individual-pollen test crosses were made on 16 western white pine (*Pinus monticola* Dougl.) trees. Seedling progenies were exposed to the white pine blister rust disease and percentages of seedlings remaining healthy in the mixed-pollen progenies were compared with percentages observed for the average of the four individual-pollen crosses made on the same trees. Ten-pollen mix cross percentages were significantly and strongly correlated with average percentages from the four crosses, and fell within ±3 percent in 19 out of 20 cases. It was concluded that for estimating general combining ability for blister rust resistance, mixed-pollen crosses (with 10 or more pollens) were an economical and reliable substitute for the more conventional but expensive multiple crosses.

Forest geneticists at several stations in the United States and abroad are using or proposing a series of three to five individual, controlled crosses for appraisal of general combining ability of plus trees. Selection of the best plus trees may be aimed at improvement of pest resistance, growth rate, stem form, branching habit, or wood-quality traits; whatever the aim, these multicross progeny tests are quite expensive. For example, in appraisal of general combining ability for blister rust resistance[3]--a relatively short-term project running only 6 to 7 years from pollination--the cost for a four-cross test has averaged $600 per tree. We need to reduce this cost before undertaking any further practical testing on a large scale.

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[1] This note is a nontechnical condensation of a research paper (Breeding blister rust resistant western white pine. IV. Mixed-pollen crosses for appraisal of general combining ability), which the author soon will submit to *Silvae Genetica*. Details of methods and analyses are given in the research paper. This summary is provided in advance because large savings in costs of progeny tests are possible through use of mixed-pollen crosses, and practical tree breeders may wish to effect these savings immediately.

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[3] Here, specifically, the transmission of above-average resistance by phenotypically resistant plus trees (rust-free but as yet untested trees from heavily infected natural stands) when pollinated with a number of similar plus trees.

One obvious way to pare the costs of progeny testing is to substitute mixed-pollen crosses for the conventional series of individual crosses. This substitution has been suggested by Goddard, Peters, and Strickland,[4] but evidence as to the relative reliability of the mixed-pollen crosses is lacking. Thus reliability criteria for mixed-pollen crosses reported here may be of general interest to practical tree breeders.

MATERIALS AND METHODS

Sixteen blister rust-resistant western white pine (*Pinus monticola* Dougl.) plus (cankerfree) trees in five heavily infected natural stands in northern Idaho were crossed as shown in table 1, using fresh pollens. Of the 16, 11 trees were crossed in 1961; 5 more were crossed in 1962.

Seed from 66 (11 X 6) test progenies were sown in fall 1962, and from 30 (5 X 6) progenies in fall 1963. A sowing design of 10 randomized blocks was used, each progeny being represented by one 16-seed plot in each block. Seedling progenies were inoculated with the blister rust fungus in 1963 and 1964 in the fall, and the final inspection for active blister rust stem lesions was made in the fall of 1965. Fourteen ordinary, nonresistant control progenies were sown--six in 1962 and eight in 1963; these were sown, inoculated, and examined in the same manner as the test progenies.

Table 1.--Controlled crosses made to check reliability of mixed-pollen crosses

Cross no.	Pollination	Term for type of cross
1	Plus tree X a single ♂ tester	Standard cross (average of four individual crosses)
2	Same plus tree X 2nd single ♂ tester	
3	Same plus tree X 3rd single ♂ tester	
4	Same plus tree X 4th single ♂ tester	
5	Same plus tree X equal-volume mix of pollens of the four testers above	4-pollen cross
6	Same plus tree X equal-volume mix of pollens of four testers above along with six other testers	10-pollen cross

RESULTS AND DISCUSSION

At the final rust examination, the average 16-seed plot contained 12.6 seedlings. Overall success of inoculation was excellent; only 3 percent of the 1962-sown and 8 percent of the 1963-sown control seedlings remained healthy (free of active blister rust cankers). Despite the high average level of infection, localized variation in level of infection was noticeable between replicates (plots) of the same plus tree or control progeny.

Percentages of healthy seedlings remaining in progenies of the three types of crosses, for each of the 16 plus trees, are shown in table 2 (columns 3, 6, and 10). The standard cross values are mean percentages (40-plot, unweighted averages), arising from four individual tester crosses made on the plus tree. (Percentages are adjusted to prevent discontinuities caused by small numbers of seedlings per plot. See Bartlett, M. S., J. Roy. Statist. Soc. Suppl. 3: 63-78, 1936.) The standard cross values are considered to give the most reliable estimates of general combining ability that we can obtain, in view of cost. Even so, they are not highly accurate, as witnessed by their relatively large standard errors (computed elsewhere, they average about one-fourth as large as the standard cross mean percentages to which they apply). From analysis of variance we know that for the standard crosses, error variance is only about one-twentieth the variance attributable to the plus trees, whereas for the two mixed-pollen crosses it amounts to almost one-fourth of that of the plus trees. This larger proportion of error variance probably reflects the much smaller number of replicates used for the mixed-pollen cross tests (10 plots vs. 40 in the standard crosses). Also, we know that the method of inoculation, which employed *Ribes* bushes, often bearing large numbers of either heavily or lightly infected leaves, resulted in wide variation in infection between plots (see footnote 1).

[4]Goddard, R. E., W. J. Peters, and R. K. Strickland. Cooperative forest genetics research program. Fourth Prog. Rep., Univ. Fla. Sch. Forest. Res. Rep. 7, 16 pp., 1962.

Table 2.--Percentages of healthy seedlings in progenies of the standard and the mixed-pollen crosses

Plus tree (1)	Standard cross: No. of trees tested (2)	Standard cross: Proportion healthy (3)	Standard cross: Ranking within best half (4)	4-pollen cross: No. of trees tested (5)	4-pollen cross: Proportion healthy (6)	4-pollen cross: Diff. from standard cross (7)	4-pollen cross: Ranking within best half (8)	10-pollen cross: No. of trees tested (9)	10-pollen cross: Proportion healthy (10)	10-pollen cross: Diff. from standard cross (11)	10-pollen cross: Ranking within best half (12)
		Percent			Percent	Percent			Percent	Percent	
1962 TEST OF 11 PLUS TREES											
61	511	10.5	5	120	13.0	+ 2.5	5	130	15.9	+ 5.4	2
69	439	11.8	4	95	13.4	+ 1.6	4	120	14.9	+ 3.1	4
70	476	7.6		113	15.2	+ 7.6	2	130	7.1	- 0.5	
224	585	4.9		149	2.2	- 2.7		147	4.4	- 0.5	
257	591	4.3		146	2.8	- 1.5		128	4.9	+ 0.6	
264	280	18.1	1	82	15.1	- 3.0	3	42	20.1	+ 2.0	1
266	534	9.0		143	12.3	+ 3.3		146	9.3	+ 0.3	
268	560	4.9		139	8.4	+ 3.5		143	3.4	- 1.5	
272	578	13.6	2	111	10.8	- 2.8		135	14.9	+ 1.3	3
276	291	13.2	3	57	20.3	+ 7.1	1	119	9.9	- 3.3	5
277	573	2.7		149	2.8	+ 0.1		133	3.6	+ 0.9	
						+25.7				+13.6	
						-10.0				- 5.8	
						+15.7				+ 7.8	
Means	492.5	9.1		118.5	10.6	+ 1.4		124.8	9.8	+ 0.7	
1963 TEST OF 5 PLUS TREES											
336	479	14.5		150	8.1	- 6.4		129	13.6	- 0.9	2
353	545	16.2	1	124	12.4	- 3.8	2	138	12.8	- 3.4	
362	491	12.0		135	10.4	- 1.6		141	10.0	- 2.0	
363	406	10.7		139	10.5	- 0.2		132	8.6	- 2.1	
367	546	14.9	2	140	14.5	- 0.4	1	121	16.8	+ 1.9	1
						+ 0.0				+ 1.9	
						-12.4				- 8.4	
						-12.4				- 6.5	
Means	493.4	13.7		137.6	11.2	- 2.5		132.2	12.4	- 1.3	
Pooled correlation coefficients for 1962 and 1963 tests (13 d.f.)[1]											
Product-moment (r)					0.785				0.919		
Rank, calculated from complete rankings (r_s)							0.879				0.956

[1]All correlation coefficients are significant at the 1-percent level of probability.

Nevertheless, table 2 shows that for most of the 16 plus trees tested, percentages of healthy seedlings found in mixed-pollen cross progenies are similar to those found in corresponding standard crosses. Closeness of the relationship is indicated by the product-moment and rank correlation coefficients given at the bottom of the table. These indicate that mixed-pollen percentages are significantly correlated with the standard cross percentages, and that the 10-pollen cross percentages ($r = 0.918$, $r_s = 0.956$) are strongly correlated.

In order to define the relative accuracy of the 4-pollen or 10-pollen crosses, however, we must resort to another sort of analysis. Here we use the specialized chi-square analysis proposed by Freese,[5] wherein accuracy of a new technique can be estimated from the difference between the results "observed" under the new technique (mixed-pollen crosses) and those "expected" under the presumably more accurate standard technique (the standard cross). An important feature of Freese's analysis is that acceptable levels of accuracy are imposed. Realizing that the standard cross values themselves were not without error, and that the practical selectionist could accept and use levels of accuracy below those desirable for critical research, we specified that to be "reliable," mixed-pollen cross values should fall within ±3 percent in the standard cross values, in 95 out of 100 cases. Another feature of the analysis is that bias--here signified by an excess of plus differences in the 1962 test, and of minus differences in the 1963 test (table 2)--can be eliminated as a cause for rejecting the new technique as inaccurate.

The chi-square analysis was performed, with percentages transformed to corresponding arc-sins as is usual with binomial (percentage) data. It showed that of the two "new techniques," only the 10-pollen cross met the established reliability criteria. This meant that there was only a 1-in-20 chance that the values of the 10-pollen cross would not fall within 3 percent of those obtained for the same plus tree by the more reliable but more expensive standard cross. For example, the 10-pollen cross value for plus tree 272 (table 2, column 10) of 14.9 percent should fall, in 95 of 100 cases, within ±3 percent of the corresponding standard cross value (column 3, 13.6 percent) for that tree, or within the range of 10.6 to 16.6 percent.

What are the implications of these findings for the practical tree breeder concerned with rust resistance? We see three implications as follows:

First. Mixed-pollen crosses which include 10 or more fresh pollens can be used to obtain economical, yet relatively reliable estimates of general combining ability. If, for example, the tree breeder were to select from table 2 the top-ranking half, or five, of the eleven plus trees of the 1962 test on the basis of the 10-pollen cross, he would choose trees 61, 69, 264, 272, and 276. Similarly, in the five-tree 1962 test he would choose two trees (336 and 367). Then, considering the standard cross results to be more reliable, he would have made only one error in his selection (choice of tree 336). Such "errors" in selection would occur only near the selection cutoff point where, with the 3-percent accuracy of the new technique, he should expect them. Furthermore, in choosing 336 as one of two trees chosen in the 1963 test, he is not 50 percent (one out of two trees) in error. Instead, near the cutoff point he has selected one tree almost, but not quite, as good, and culled one almost, but not quite, as poor as the trees he would have selected using the standard crosses.

Second. Wide variation in intensity of inoculation and infection between plots should be reduced, or the number of plots (and thus the cost) increased in mixed-pollen cross testing. We intend to concentrate on securing uniform inoculation--by random placement of rust-infected *Ribes* spp. leaves, not bushes, over the small plots in the test nursery.

Third. Although the mixed-pollen crosses are economical, the breeder using them must be willing to sacrifice (a) some accuracy in selection, and (b) the recognition of specific combining ability.[6] By using 10 or more pollens in the mixed-pollen crosses, along with the improved inoculation techniques, we expect to lower testing costs from about $600 to $150 per selection.

[5] Freese, Frank. Testing accuracy. Forest Sci. 6(2): 139-145. 1960.

[6] The ability of specific, resistant plus trees to transmit above-average resistance to their offspring, but only when mated with certain other specific, resistant plus trees.